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14. ABSTRACT This purpose of this proposal is to use high resolution microPET technology to image ovarian cancer integrin $\alpha v\beta 3$ expression in vivo. Ovarian cancer is angiogenesis dependent. Integrin $\alpha v\beta 3$, a key player in tumor angiogenesis and metastasis, has been identified as a target for diagnostic and therapeutic interventions for several highly proliferative and metastatic tumor types. Specific Aim 1: To develop and optimize 18F-labeled RGD peptides for ovarian carcinoma targeting. Specific Aim 2: To test 18F-RGD peptide tracers in ovarian carcinoma models of different tumor integrin $\alpha v\beta 3$ expression levels in order to correlate the magnitude of tumor uptake with receptor density. Major Findings: In year 1, we have synthesized a series of multimeric RGD peptides with high integrin $\alpha v\beta 3$ affinity/specificity and labeled these peptides with F-18 for PET imaging of integrin expression in vivo (Aim 1). In year 2, we have also established several ovarian cancer models with differentiated integrin levels (Aim 2). One of the dimeric RGD peptide tracer 18F-FPRGD2 with high tumor targeting efficacy and favorable in vivo kinetics has been subjected to GLP toxicity study. Exploratory IND (eIND) application is currently underway and first-in-human studies are being planned. Further test of the optimal radiotracer in different ovarian cancer models to correlate the tracer uptake with tumor integrin expression is currently underway as a non-cost of extension (Aim 2).

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INTRODUCTION

Ovarian epithelial carcinomas are characterized by local proliferation and invasion of ovarian surface epithelium (OSE)-derived tumor cells, followed frequently by dissemination in the peritoneal cavity and formation of ascites containing clusters of tumor cells. This cancer is therefore characterized by the presence of two forms of call populations, some growing as solid tumors and others in suspension (*1*). At each step of malignant progression, adhesive mechanisms plays an important role, and integrins are likely to participate in these events (*2-8*). In most ovarian cancer cells, integrin $\alpha_v\beta_3$ is expressed and its ligand vitronectin is synthesized as a primary adhesion substrate. In fact, integrin $\alpha_v\beta_3$ is found at a significantly higher rate in primary ovarian cancer than in ovarian tumors of low malignant potential, a fact which points to a role of $\alpha_v\beta_3$ during ovarian cancer progression. Although the precise mechanism of tumor progression promoted via $\alpha_v\beta_3$ is still rather inconclusive, various studies support a potential role of $\alpha_v\beta_3$ in selective invasion and the generation of growth and survival signals (*9-12*). A recent study evidenced the correlation between α_v-integrin expression and poor survival in ovarian carcinoma (*11*).

The ability to non-invasively visualize and quantify $\alpha_v\beta_3$ integrin expression level will provide new opportunities to document tumor (tumor cells and sprouting tumor vasculature) receptor expression, more appropriately select patients considered for anti-integrin treatment and monitor treatment efficacy in integrin-positive patients (*13, 14*). In the past few years, we have developed a series of peptide and antibody based probes for multimodality imaging of integrin expression in vivo. This annual report will only summarize the progress highly relevant to this OCRP pilot proposal. We hypothesize that ovarian cancer integrin expression can be visualized and quantified with suitably labeled RGD peptide antagonist of integrin $\alpha_v\beta_3$ and that the magnitude of tumor uptake from non-invasive molecular imaging may be well-correlated with tumor integrin expression levels.

BODY

Chemistry and Radiochemistry

During year 1 of the funding period, we have reported the development of RGD monomer, dimer, and tetramer labeled with ^{18}F using N-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB) as the synthon. However, this procedure suffers from lengthy and tedious multistep synthetic procedures. As a result, it is challenging to automate and adversely decrease the overall radiolabeling yield. Nevertheless, the lead compound ^{18}F-FB-mini-PEG-E[c(RGDyK)]$_2$ (^{18}F-FPRGD2) is currently being translated into clinic for first-in-human studies.
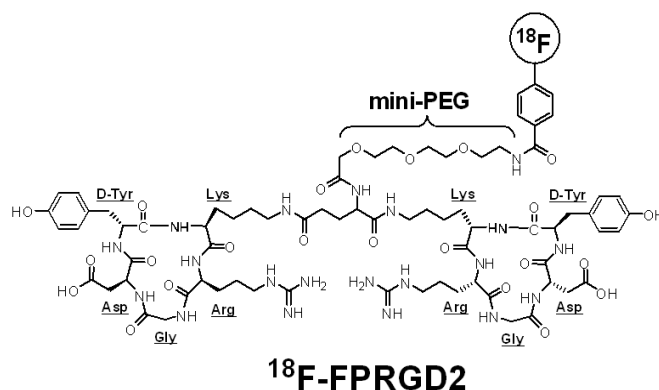

Fig. 1. Schematic structure of ^{18}F-FPRGD2.

The single species acute toxicity study has been done in collaboration with the Division of Veterinary Medicine and Surgery, the University of Texas M.D. Anderson Cancer Center (Table 1). No observed adverse effect was found. An exploratory IND is being prepared and will be submitted to FDA for phase 0 studies.

Table 1. Acute toxicity study of ^{18}F-FPRGD2 in Sprague-Dawley rats.

| Group Number | Number of Animals | | Euthanasia (Study Day) | Necropsy Procedures | | | Histology/ Histopathology |
	Male	Female		Necropsy	Tissue Collection	Organ Weights	
I	5	5	1	C	C	X	C
II	5	5	1	C	C	X	C
I	5	5	14	C	C	X	C
II	5	5	14	C	C	X	C
Found Dead or Accidentally Killed	As appropriate			C	C	NA	C (tissue permitting)
Moribund				C	C	NA	C

The recent discovery that Cu(I) catalyzes the Huisgen 1,3-dipolar cycloaddition of organo azides with terminal alkynes to form 1,2,3-triazoles (*15, 16*), often referred to as 'click chemistry' (*17*), has lead to wide applications in combinatorial chemistry (*18-20*). This reaction could be carried out in high yields under mild conditions, and the 1,2,3-triazole formed has similar polarity and size with amide bond (*21*). Due to these favorable aspects with 'click chemistry', the use of this reaction for making ^{18}F labeled RGD peptides have been successfully conducted in our lab.

As shown in Fig. 1, both alkyne-tosylate (**1**) and azido-RGD2 were obtained in high yields. The alkyne-fluoride was prepared in situ and could be used directly for the reaction with azido-RGD2 to make the cold standard, which was purified by HPLC and confirmed by MALDI-TOF mass spectrometry. The radiochemical purity of the ^{18}F-labeled peptide ^{18}F-FPTA-RGD2 was higher than 97% according to analytical HPLC. The specific radioactivity of ^{18}F-FPTA-RGD2 was determined to be about 100–200 TBq/mmol, as the unlabeled azido-RGD2 was efficiently separated from the product.
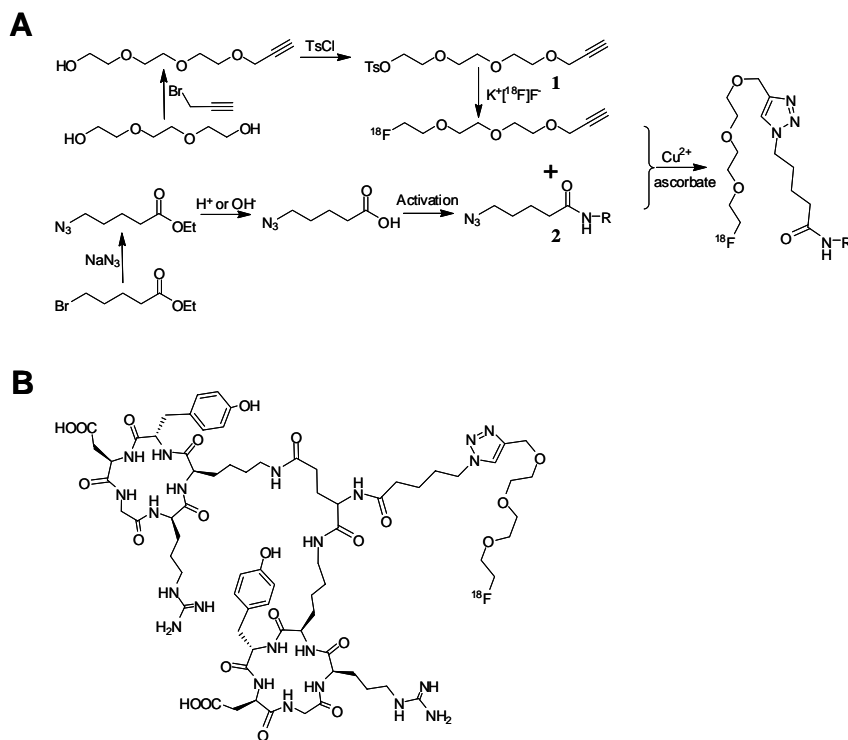

Figure 1. (A) Radiosynthesis of ^{18}F-fluoro-PEG-alkyne intermediate and 1.3-dipolar cycloaddition with terminal azide. R = targeting biomolecule (peptides, proteins, antibodies et al.). (B) Structure of ^{18}F-fluoro-PEG-alkyne labeled E[c(RGDyK)]$_2$: ^{18}F-fluoro-PEG-triazole-E(RGDyK)$_2$ (^{18}F-FPTA-RGD2).

The octanol/water partition coefficient (logP) for ^{18}F-FPTA-RGD2 was -2.71$\pm$0.006, indicating that the tracer is slightly more hydrophilic than [18]F-FB-RGD2 ([18]F-FRGD2, -2.103$\pm$0.030) and [18]F-FB-PEG3-RGD2 ([18]F-FPRGD2, -2.280$\pm$0.054) (*22*).

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In vitro Cell Integrin Receptor-Binding Assay

The receptor-binding affinity of RGD2 and FPTA-RGD2 was determined by performing competitive displacement studies with ^{125}I-echistatin. All peptides inhibited the binding of ^{125}I-echistatin (integrin $\alpha_v\beta_3$ specific) to U87MG cells in a concentration dependent manner. The IC_{50} values for RGD2 and FPTA-RGD2 were 79.2 ± 4.2 and 144 ± 6.5 nM, respectively (n = 3) (Fig. 2). In a parallel experiment, the IC_{50} value for FPRGD2 was 97 ± 4.8 nM. The comparable IC_{50} values of these compounds suggest that the introduction of miniPEG linker and triazole group had little effect on the receptor binding affinity.
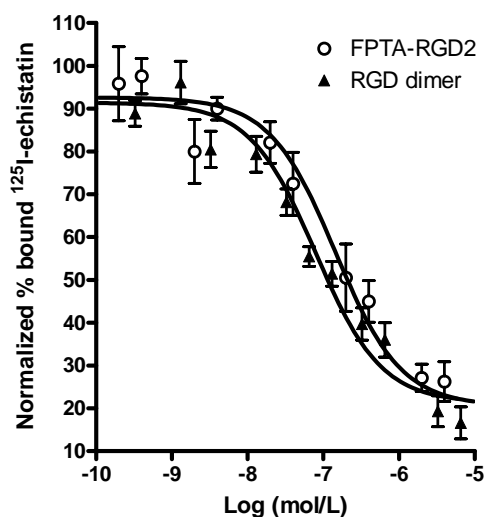

Figure 2. Cell binding assay of E[c(RGDyK)]$_2$ and FPTA-RGD2 using U87MG cells with competitive displacement studies using ^{125}I-echistatin. The IC_{50} values for E[c(RGDyK)]$_2$ and FPTA-RGD2 were 79.2 ± 4.2 and 144 ± 6.5 nM, respectively (n = 3).

microPET Imaging Studies

Dynamic microPET scans were performed on U87MG xenograft model and selected coronal images at different time points after injecting ^{18}F-FPTA-RGD2 were shown in Figure 3A. Good tumor-to-contralateral background contrast was observed as early as 10 min after injection (5.4 ± 0.7 %ID/g). The U87MG tumor uptake was 3.1 ± 0.6, 2.1 ± 0.4, and 1.3 ± 0.4 %ID/g at 0.5, 1, and 2 h p.i., respectively (n = 3). Most activity in the non-targeted tissues and organs were cleared by 1 h p.i. For example, the uptake values in the kidney, liver, and muscle were as low as 2.7 ± 0.8, 1.9 ± 0.4, and 1.0 ± 0.3 %ID/g, respectively at 1 h p.i. ^{18}F-FPTA-RGD2 was cleared mainly through the kidneys. Some hepatic clearance was also observed. The integrin $\alpha_v\beta_3$ specificity of ^{18}F-FPTA-RGD2 in vivo was confirmed by a blocking experiment where the tracer was co-injected with c(RGDyK) (10 mg/kg). As can be seen from Figure 3B, the U87MG tumor uptake in the presence of non-radiolabeled RGD peptide (0.9 ± 0.3 %ID/g) is significantly lower than that without RGD blocking (2.1 ± 0.4 %ID/g) ($P < 0.05$) at 1 h p.i.
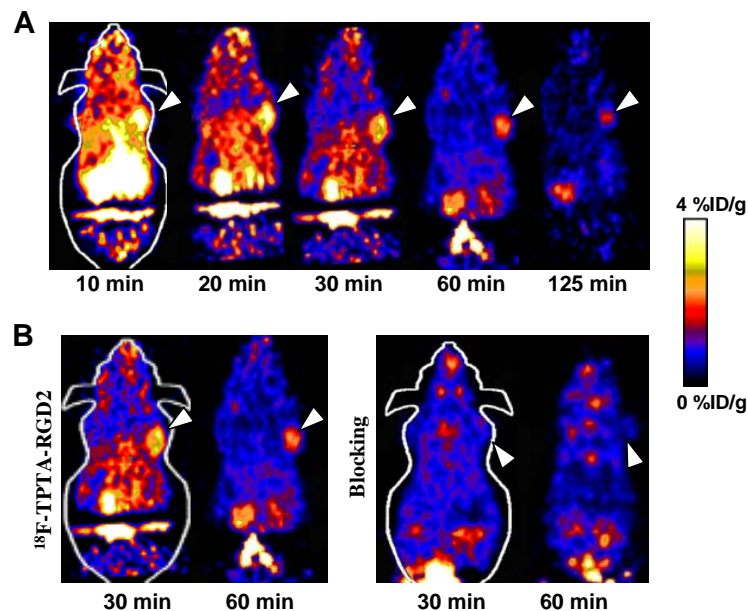
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Figure 3. (A) Decay-corrected whole-body coronal microPET images of athymic female nude mice bearing U87MG tumor at 10, 20, 30, 60 and 125 min post-injection (p.i.) of about 2 MBq of ^{18}F-FPTA-RGD2. (B) Coronal microPET images of U87MG tumor-bearing mice at 30 and 60 min p.i. of ^{18}F-FPTA-RGD2 with (denoted as "Blocking") and without coinjection of 10 mg/kg mouse body weight of c(RGDyK). Tumors are indicated by arrows.
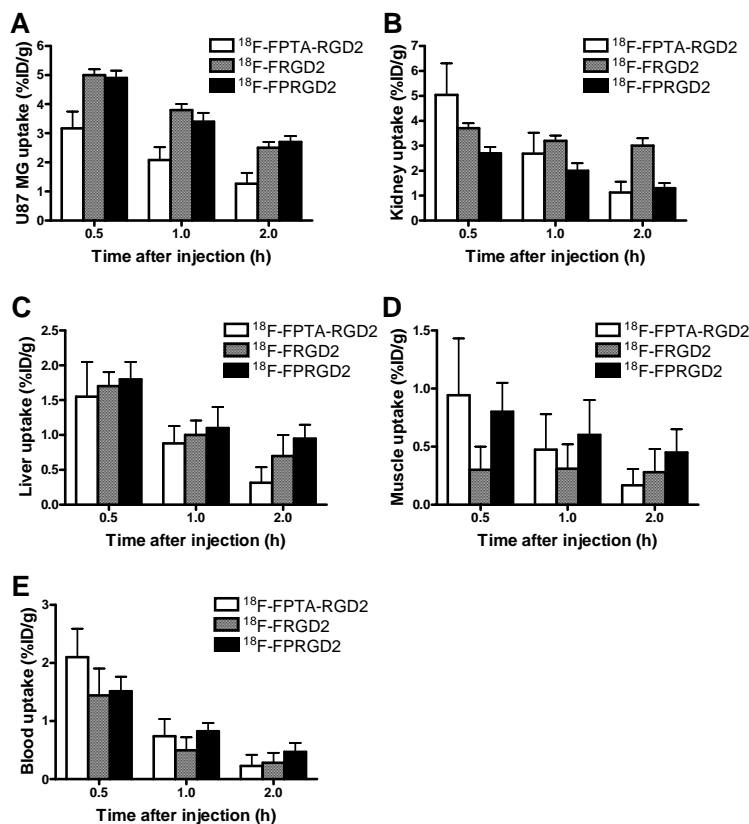
Figure 4. Comparison of ^{18}F-FPTA-RGD2, ^{18}F-FB-RGD2 (^{18}F-FRGD2) and ^{18}F-FB-PEG3-RGD2 (^{18}F-FPRGD2) in U87MG tumor, kidney, liver, muscle, and blood over time.

The comparison of tumor and various organ uptake of ^{18}F-FPTA-RGD2 with ^{18}F-FPRGD2 and ^{18}F-FRGD2 were shown in Figure 5. The uptake in the U87MG tumor was slightly lower for ^{18}F-FPTA-RGD2 which might be caused by integrin $\alpha_v\beta_3$ binding affinity difference (Fig. 4A). The kidney uptake for these three tracers was comparable (Fig. 4B) and the clearance rate was highest for ^{18}F-FPTA-RGD2. ^{18}F-FPTA-RGD2 had lowest liver uptake which was consistent with the hydrophilic sequence of these three compounds (Fig. 4C). The non-specific uptake in the muscle was at a very low level for all three compounds (Fig. 4D).

In Vivo Metabolic Stability Studies

The metabolic stability of ^{18}F-FPTA-RGD2 was determined in mouse blood and urine and the in liver, kidney and tumor homogenates at 1 h after intravenous injection of radiotracer into a U87MG tumor-bearing mouse. The extraction efficiency of all organs was between 86% and 99% (Table 2). The lowest extraction efficiency was found for the kidney. There are 1% to 41% of the total activity could not be trapped on the C-18 cartridges, which can be related to very hydrophilic metabolites and protein-bound activity. After ACN elution, the radioactivity of each sample was injected onto an analytical HPLC and the HPLC chromatograms are shown in Figure 6. The fraction of intact tracer was between 75 % and 99 % (Table 2). Although we did not identify the metabolites, we found that all metabolites eluted earlier from the HPLC column than the parent compound (Fig. 5), which behaved similarly to ^{18}F-FRGD2 (*23*) and ^{18}F-FPRGD2 (*22*).

Table 2. Extraction efficiency, elution efficiency, and HPLC analysis of soluble fraction of tissue homogenates at 1 h post-injection of ^{18}F-FPTA-RGD2.

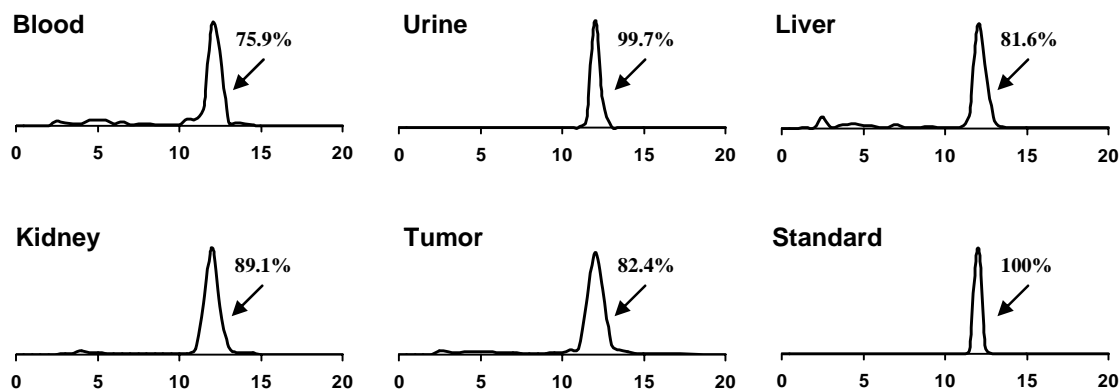| Fraction	Blood	Urine	Liver	Kidney	U87MG
Extraction efficiency (%)					
Insoluble fraction	0.8	ND	10.3	13.3	7.5
Soluble fraction	99.2	ND	89.7	86.7	92.5
Elution efficiency (%)					
Unretained fraction	2.8	0.4	33.9	12.8	18.5
Wash water	8.8	0.5	7.4	3.9	5.2
Acetonitrile eluent	88.4	99.1	58.7	83.3	76.4
HPLC analysis (%)					
Intact tracer	75.9	99.7	81.6	89.1	82.4

Figure 5. Metabolic stability of ^{18}F-FPTA-RGD2 in mouse blood and urine samples and in liver, kidney and U87MG tumor homogenates at 1 h after injection. The HPLC profile of pure ^{18}F-FPTA-RGD2 (Standard) is also shown.

KEY ACCOMPLISHMENTS

- Completed single-dose acute toxicity study of ^{18}F-FPRGD2 preparing for eIND application and first-in-human test;
- Develop a novel click chemistry strategy to label RGD peptides with ^{18}F with shorter reaction time and easier chemistry than traditional ^{18}F-SFB synthon;

REPORTABLE OUTCOMES

Publications:

Wu Z, Li Z-B, Cai W, Chin FT, Li F, <u>Chen X</u>
^{18}F-labeled mini-PEG spacered RGD dimer (^{18}F-FPRGD2): synthesis and microPET imaging of $\alpha_v\beta_3$ integrin expression
Eur J Nucl Med Mol Imaging 2007;34(11):1823-1831.

Wu Z, Li Z-B, Cai W, He L, Chin FT, Li F, <u>Chen X</u>
microPET Imaging of Tumor $\alpha_v\beta_3$ Integrin Expression Using ^{18}F-labeled PEGylated Tetrameric RGD Peptide (^{18}F-FPRGD4)
J Nucl Med, 2007;48:1536-44.

Li ZB, Wu Z, Chen K, Chin FT, <u>Chen X</u>.
Click Chemistry for ^{18}F-Labeling of RGD Peptides and microPET Imaging of Tumor Integrin alphavbeta3 Expression
Bioconjug Chem 2007;18(6):1987-1994.

Conference Abstracts:

Li Z-B, Wu Z, Chen K, Chin FT, <u>Chen X</u>
Click Chemistry for ^{18}F-Labeling of RGD Peptides and microPET Imaging of Tumor $\alpha v\beta 3$ Integrin Expression
Joint Molecular Imaging Conference, Providence, Rhode Island, September 2007

CONCLUSIONS

In conclusion, we have developed a novel click chemistry strategy to label dimeric RGD peptide for tumor integrin expression. We have also completed the GLP toxicity studies of ^{18}F-FPRGD2 and an eIND application is to be filed very soon for first-in-human studies. Due to the unexpected leave of a stellar postdoctoral fellow for a tenure-track faculty position, this project is somewhat delayed. The correlation of tumor uptake and receptor density in ovarian cancer was not finished by the end of the 2nd year funding period. A no-cost-extension (NCE) was filed and a new postdoc has been trained to continue the project and a final report will be submitted by the end of the NCE period.

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APPENDICES

N/A